Modulation of Casein Kinase II Activity by the Polar Head Group of an Insulin-sensitive Glycosyl-phosphatidylinositol*

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A phospho-oligosaccharide, whose production is stimulated by insulin, modulated the activity of partially purified casein kinase II. Whereas at 2 μM the phospho-oligosaccharide stimulated casein kinase II 1.3-fold, higher concentrations of this molecule were inhibitory. 50% inhibition of the enzyme was obtained at 15 μM phospho-oligosaccharide. This biphasic effect of the phospho-oligosaccharide on casein kinase II activity was observed using as substrate both casein or the specific peptide for casein kinase II, Arg-Arg-Arg-Glu-Glu-Thr-Glu-Glu-Glu. The effect of the phospho-oligosaccharide on casein kinase II was still observed after gel filtration. Deamination of the phospho-oligosaccharide with nitrous acid abolished both the activation and the inhibition of casein kinase II. The glycoprophospholipid precursor of the phospho-oligosaccharide did not affect casein kinase II activity. Moreover, modulation of casein kinase II activity was not observed with other compounds structurally related to the phospho-oligosaccharide, when used in the micromolar range. In conclusion, the present results indicate that the phospho-oligosaccharide that mimics and might mediate some of the actions of insulin modulates casein kinase II activity in vitro.

A glycosyl-phosphatidylinositol has been implicated in insulin action. It has been shown that insulin promotes the hydrolysis of a glycosyl-phosphatidylinositol releasing its polar head group (POS)\(^1\) that contains phosphoinositol, glucosamine, 4 galactose residues, and an average of 2 additional phosphates, probably attached to a residue of galactose (5, 6). In hepatocytes, the glycosyl-phosphatidylinositol is mostly located at the outer surface of the cell (7), and there is evidence indicating that the extracellular concentration of POS increases after the addition of insulin to intact cells (8). POS has been reported to elicit some of the biological effects of insulin when added to intact cells. It faithfully copies the insulin-directed effects on protein phosphorylation/dephosphorylation (9, 10), inhibits phospholipid methytransferase and lipolysis and stimulates lipogenesis in adipocytes, and reduces glycogen phosphorylase and cAMP levels and stimulates pyruvate kinase in hepatocytes (11–15). Furthermore, this molecule modulates several enzymatic activities when added to cell extracts (1, 14, 16) and inhibits purified cAMP-dependent protein kinase but not protein kinase C (17).

Casein kinase is a widely distributed serine/threonine protein kinase that is able to phosphorylate many different substrates (for a review, see Refs. 18 and 19), including itself (20). Casein kinase II activity is enhanced during differentiation of 3T3-L1 mouse adipocytes (21) and N2A mouse neuroblastoma cells (22). It has been shown that serum and growth factors regulate casein kinase II activity (23–25). Insulin and epidermal growth factor activate casein kinase II activity 1.3-fold in 3T3-L1 mouse adipocytes and H4-IIE cells (24). Insulin and insulin-like growth factor-I, but not epidermal growth factor, increase ~2-fold the casein kinase activity in BALB/c 3T3 fibroblasts (25). The mechanism by which these hormones modulate casein kinase II is unknown, and cAMP, cGMP, Ca\(^{2+}\)/calmodulin, and Ca\(^{2+}\)/phospholipid have been excluded as modulators of this enzyme (18, 19). Casein kinase II is activated by polycations and inhibited by polyamines (18, 19, 26), although the physiological significance of these effects remain to be determined. In this paper we report that partially purified rat liver casein kinase II is regulated by micromolar concentrations of POS.

**EXPERIMENTAL PROCEDURES**

**Partial Purification of Casein Kinase II Activity—**Three rats (200 g weight) were decapitated and the livers excised and placed on ice. All subsequent operations were performed at 0–4 °C. The livers were chopped and homogenized in 4 volumes of 0.3 M sucrose, 10 mM Tris/HCl pH 7.4 at 20 °C, 0.1% (v/v) 2-mercaptoethanol, and the proteinase inhibitors benzamidine (B-209, Sigma) (1 mM) and phenylmethylsulfonyl fluoride (P-7626, Sigma) (0.1 mM). The homogenate was filtered after a double layer of gauze and centrifuged for 15 min at 6000 × g and the supernatant (the cytosolic fraction) was loaded on a DEAE-Sephasel (Pharmacia LKB Biotechnology Inc.) column (5 × 3 cm) equilibrated in 100 mM Tris/HCl pH 7.5 at 25 °C, containing 0.1% (v/v) 2-mercaptoethanol and proteinase inhibitors. The column was washed with 50 ml of this buffer and a linear gradient was developed from 0 to 500 mM NaCl. Casein kinase II activity eluted between molecular size markers β-amylase (A-8781, Sigma) (200 kDa) and alcohol dehydrogenase (A-8656, Sigma) (150 kDa).

**Assay of Casein Kinase II Activity—**Casein kinase activity was assayed for 30 min at 30 °C when casein (C-4765, Sigma) was used as substrate, or for 20 min when a specific substrate for casein kinase II (Arg-Arg-Glu-Glu-Thr-Glu-Glu, Peninsula Laboratories, Inc.) was used as substrate. The reactions were linear for at least 30 min, or for 20 min when a specific substrate for casein kinase II was used.

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\(^1\) The abbreviations used are: POS, phospho-oligosaccharide; DPAL, propyl-P-galactoside 2,6-bisphosphate; DPME, methyl-n-galactoside 2,6-bisphosphate.
treatment of POS with nitrous acid (Table II). Half-maximal inhibition of casein kinase II activity was observed at 15 μM POS (Fig. 1). DPAL and DPME, two different galactose bisphosphate compounds, were not able to affect casein kinase activity when used from 1 to 500 μM (data not shown). At millimolar concentrations, however, both compounds inhibited casein kinase II activity (Fig. 2). The half-maximal inhibition of kinase activity was observed at 3 and 2 mM, respectively (Fig. 3), these data are in agreement with the values previously reported for both substrates using purified casein kinase II (31, 32). The inhibitory effect of 15 μM POS on casein kinase II activity was the result of a reduction of the $V_{max}$ without affecting the $K_m$ of the enzyme (Fig. 3). In addition, the inhibitory effect of POS on casein kinase II was independent of the concentration of both casein and ATP (Fig. 3).

Casein kinase II activity is modulated by polyanions and polycations (18, 19, 26). Fig. 4 shows the influence of different concentrations of POS (2, 6, 10, and 30 μM) on the effect of heparin (60 ng/ml), DPAL (1 mM), DPME (1 mM), and polylysine (40 and 200 μM) on casein kinase II. DPAL (1 mM) and POS (6 μM) inhibited casein kinase II activity by 40 and 8%, respectively, and when added together produced an inhibi-
Modulation of Casein Kinase II Activity

Fig. 2. Effects of increasing concentrations of DPAL or DPME on casein kinase II activity. Assays contained from 0 to 7 mM of DPAL (●) or DPME (○), and incubations were carried out as described under "Experimental Procedures" using casein as substrate. Results are the mean ± S.D. from two different experiments carried out in triplicate.

![Graph showing the effects of different concentrations of DPAL and DPME on casein kinase II activity.](image)

Fig. 3. Lineweaver-Burk plots for the inhibition of casein kinase II by 15 μM POS. A, casein concentration was varied in the assay from 0.1 to 4 mg/ml, and incubations were carried out as described under "Experimental Procedures." Results are the mean ± S.D. from three different experiments carried out in triplicate. B, ATP was varied from 5 to 500 μM, and incubations were carried out as described under "Experimental Procedures" using casein as substrate. Results are the mean ± S.D. from three experiments carried out in triplicate. ○, control; ●, 15 μM POS.

![Graph showing Lineweaver-Burk plots.](image)

Table III

Effect of gel filtration on POS modulation of casein kinase II activity

<table>
<thead>
<tr>
<th>Addition</th>
<th>Casein kinase activity % control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>POS 2 μM</td>
<td>120 ± 17</td>
</tr>
<tr>
<td>POS 20 μM</td>
<td>60 ± 25</td>
</tr>
</tbody>
</table>

The effects of POS on casein kinase II activity were still observed after gel filtration of the enzyme through a Bio-Gel P-6 column. Thus, when casein kinase II was incubated in the presence of 2 μM POS and subjected to gel filtration, an activation of the enzyme was still observed (Table III). Similarly, when casein kinase II was incubated in the presence of 20 μM POS and subjected to gel filtration, an inhibition of the kinase activity was observed (Table III). The reason for the relatively large dispersion of these data, specially when

![Graph showing the influence of POS on casein kinase II activity.](image)
the enzyme was incubated in the presence of 2 μM POS, is not known. Under the present conditions for gel filtration, about 99% of [3H]galactose-labeled POS was resolved from the kinase and no significant radioactivity was found associated with the peak of protein, indicating that the concentration of free POS during the assay of the enzyme would not be higher than 0.2 μM.

**DISCUSSION**

The present results indicate that partially purified rat liver casein kinase II was modulated by POS. The effects of POS on casein kinase II were observed using as substrates both casein and a specific peptide, and were independent of the concentration of both casein and ATP. These data indicate that POS interacts with the protein kinase rather than with the enzyme substrates. Although casein kinase II activity is affected by the presence of polycations and polyanions in the incubation media, the modulation of casein kinase II by POS cannot be explained only by the presence of phosphate groups in this molecule. Thus, glycosyl-phosphatidylinositol, the precursor of POS, was not able to reproduce the effects of POS on casein kinase II activity at concentrations at which POS was active. Similarly, DPAL and DPME, two galactose bis-phosphate derivatives, only inhibited casein kinase II at a concentration 100-fold higher than that of POS, and inositol 1,4,5-trisphosphate had no effect on casein kinase II activity when assayed at micromolar concentrations (data not shown). These results, together with the observation that the effects of POS were at least partially additive with those of heparin, DPME, DPAL, and polylysine, and that the activity of POS was abolished **after nitrous acid deamination**, strongly indicate that the effects of this molecule on casein kinase II are specific. The observation that the effects of POS on casein kinase II are still observed after gel filtration suggest the existence of a high affinity interaction between POS and the enzyme, although the possibility that POS causes a stable modification of the enzyme cannot be excluded. The finding that labeled POS is resolved from the kinase does not exclude the possibility that a small amount of this molecule is tightly bound to the kinase.

Casein kinase II is not the only kinase that has been shown to be modulated by POS in vitro. cAMP-dependent protein kinase is inhibited by POS with a half-maximal inhibition of 2 μM POS (17), a concentration at which POS produces a small but reproducible stimulation of casein kinase II. Although it is known that POS is generated in response to the cell differentiation (21, 22) and that insulin and epidermal growth factor-I, but not epidermal growth factor or the platelet-derived growth factor, stimulate casein kinase II (25). The mechanism(s) by which these various signals stimulate casein kinase II is at present unknown, but it is interesting to note that in both cell systems the activation was still observed after partial purification of the enzyme (24, 25). It has been reported that, in addition to insulin, epidermal growth factor and insulin-like growth factor-I also stimulate glycosyl-phosphatidylinositol hydrolysis (33). These results raise the interesting possibility that the effect of insulin and other signals on casein kinase II might be mediated by POS.

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